

## Determination of changes in antioxidant potential of different plant infusions during storage

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### Abstract

This study investigated the changes in total phenolic content (TPC), total flavonoid content (TFC), free radical scavenging capacity (FRSC), iron chelating capacity (ICC) values of cinnamon bark, linden flower, linden leaf, sage leaf, and sage stem infusions during storage. The infusions were prepared at 75°C, 85°C, and 95°C for 30 minutes and stored at + 4°C and - 18°C for 30 days. The measurements were carried out on the 5th, 15th, and 30th day of storage. The variations of the determined parameters between the storage days were observed. In cinnamon bark infusion, TPC, TFC, FRSC, ICC values decreased during storage whereas TPC, FRSC, and ICC of sage stem infusions increased. In linden flower, linden leaf, and sage leaf infusions, the common variations were observed as TPC slightly decreased, TFC decreased, FRSC and ICC did not considerably change. These general observations were obtained for every studied infusion and storage temperature of the herbal teas. This study revealed that homemade herbal teas have the potential to be good antioxidants and phenolic sources for human consumption, and they can be stored when they chilled or frozen in the refrigerator.

**Keywords:** Cinnamon; Linden; Sage; Infusion; Storage; Antioxidant

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## Depolama sırasında farklı bitki çaylarının antioksidan potansiyelindeki değişikliklerin belirlenmesi

### Öz

Bu çalışmada tarçın kabuğu, ıhlamur çiçeği, ıhlamur yaprağı, adaçayı yaprağı ve adaçayı sapı infüzyonlarının (çaylarının) depolama sırasında toplam fenolik içerik (TPC), toplam flavonoid içerik (TFC), serbest radikal süpürme kapasitesi (FRSC) ve demir şelatlama kapasitesi (ICC) değerlerindeki değişimler incelenmiştir. İnfüzyonlar 75°C, 85°C ve 95°C'de 30 dakika süreyle hazırlanmış ve +4°C ve -18°C'de 30 gün süreyle saklanmıştır. Ölçümler depolamanın 5., 15. ve 30. günlerinde gerçekleştirilmiştir. Belirlenen parametrelerin depolama günleri arasındaki değişimleri gözlemlenmiştir. Tarçın kabuğu infüzyonunda TPC, TFC, FRSC, ICC değerleri depolama süresince azalırken, adaçayı sapı infüzyonlarının TPC, FRSC ve ICC değerleri artmıştır. Ihlamur çiçeği, ıhlamur yaprağı ve adaçayı yaprağı infüzyonlarında, TPC hafifçe azalırken, TFC'de belirgin azalışlar tespit edilmiş FRSC ve ICC önemli ölçüde değişmemiştir. Bu genel gözlemlerin, çalışılan bitki tipi ve depolama sıcaklığı için geçerli olduğu görülmüştür. Bu çalışma, ev yapımı bitki çaylarının insan tüketimi için iyi bir antioksidan ve fenolik madde kaynağı olma potansiyeline sahip olduklarını ve soğutularak veya dondurularak uzun süre saklanabileceklerini ortaya koymuştur.

**Anahtar Kelimeler:** Tarçın; Ihlamur; Adaçayı; İnfüzyon; Antioksidan; Depolama

## 1. Introduction

Herbal teas produced by infusion or decoction from aromatic and medicinal plants or spices have been consumed for medicinal purposes flavorings and refreshments since the ancient times [1–4]. Many studies revealed that most of those medicinal herbs or spices showed considerable antioxidant activity by their phenolics, vitamins and alkaloids contents [5, 6]. The antioxidant compounds protect the human metabolism from the effects of free radicals, peroxy radicals, hydroxyl radicals, singlet oxygens and superoxide anions that damages DNA, protein, and lipid structures by oxidation reactions [7–9]. Therefore, those molecules do not show their functions properly, and the risk of occurrence of diseases such as cancer, atherosclerosis, neurodegenerative diseases increases [10, 11]. Due to those beneficial effects, natural phytochemicals extracted from leaves, stems, or flowers of herbal plants have been used for therapeutical purposes. Especially phenolic compounds attracted notable attention, which were mostly found in complex form with other phenolic compounds or other polymeric carbohydrate or lignin in plant tissues. This situation restricts the extraction of phenolic compounds in soluble form from plants. To overcome this problem, thermal extraction processes or organic solvent usage were applied. However, the usage of organic solvents is not preferred for the extracts that would be consumed by humans because of the toxicity of those solvents. Therefore, additional steps are needed to eliminate the solvent from the extract.

The hot water extraction is more appropriate because it softens and disrupts the cellular material and polymeric structures, which allows phenolic compound release to the medium. On the other hand, thermal application could have the detrimental effect on heat labile phenolic compounds that lower the antioxidant capacity of the extracts. Another crucial factor affecting the antioxidant activity of extracts is storage conditions. Especially, the antioxidant potential of herbal infusions might decrease during storage depending on the conditions such as temperature, light, and humidity [12]. For this reason, it is essential to measure the stability of soluble phenolic compounds and antioxidant activity of herbal infusions.

Among those herbal materials, cinnamon (*Cinnamomum verum*), sage (*Salvia officinalis*) and linden (*Tilia cordata*) are the ones mostly used for tea preparation due to their good aroma, pleasant flavor, and health benefits. In the literature, many studies reported their high antioxidant potential sourced from their phenolic compounds [3, 13–16]. However, no studies in the literature were found to have investigated the stability of those infusions at cold and freezing storage conditions, which were the simplest applications for industry and home consumers. For this reason, this study aimed to determine the effects of cold (+4°C) and freezing (-18°C) storage conditions on soluble phenolic content, soluble flavonoid content, free radical scavenging capacity, iron chelating activity, pH, and color properties of cinnamon bark, linden flower, linden leaf, sage leaf, and sage stem infusions prepared at 75, 85, and 95°C.

## 2. Methods

### 2.1. Materials

The dry cinnamon bark (*Cinnamomum verum*), sage (*Salvia officinalis*), and linden (*Tilia cordata*) were purchased from local market. Methanol and Folin Ciocalteu reagent were purchased from Merck (Germany), DPPH, ABTS, trolox, FeCl<sub>2</sub>, and Ferrozine® were purchased from Sigma Aldrich (the USA), and Na<sub>2</sub>CO<sub>3</sub>, NaNO<sub>2</sub>, and AlCl<sub>3</sub> were purchased from Isolab (Turkey). All other chemicals used in the analysis were at analytical grade.

### 2.2. Preparation of herbal infusions

The separated leaf and stem part of sage, leaf and flower part of linden, and cinnamon bark were brewed as 5 g herbal sample in 100 ml deionized water at 75 °C, 85 °C and 95 °C. Then infusions were stored at +4°C and -18°C for 30 days and the measurements were conducted at 0<sup>th</sup>, 6<sup>th</sup>, 15<sup>th</sup> and 30<sup>th</sup> days of storage.

### 2.3. Total phenolic content (TPC) of herbal infusions

After incubation of 100 µl of the herbal infusion mixed 1000 µl of Folin Ciocalteu reagent (diluted 1/10) for 3 minutes, 800 µl of 7.5% (w/v) Na<sub>2</sub>CO<sub>3</sub> was added to the mixture and further incubated for 2 hours in the dark [17].

Then the formed color was measured spectrophotometrically at 765 nm and the results were expressed as gallic acid equivalent as average of 3 parallels (Agilent Carry 60, USA).

#### **2.4. Total flavonoid content (TFC) of herbal infusions:**

Firstly, 250 µl herbal infusion diluted with 1000 µl distilled water was mixed with 75 µl 5% (w/v) NaNO<sub>2</sub> and incubated for 5 minutes [18]. Then 75 µl of 10% (w/v) AlCl<sub>3</sub> was added into the mixture and left for 1 minute. The reaction stopped by adding 500 µl 1 mol/L NaOH and mixture was diluted with 600 µl distilled water. The formed color measured spectrophotometrically at 510 nm and the results were expressed in quercetin equivalent as an average of 3 parallels.

#### **2.5. Free Radical Scavenging Capacity (FRSC) of Herbal Infusions:**

Firstly, infusions prepared in 50 µl of 80% (v/v) methanol solution were mixed with 1.95 ml DPPH radical solution (the initial absorbance value of the DPPH solution is  $0.700 \pm 0.010$  at 517 nm and left for 30 minutes incubation in the dark [19]. Then the decrease in the absorbance of the mixture was determined and the results were given as Trolox equivalent as average of 3 parallels.

#### **2.6. Iron chelating Capacity (ICC) of Herbal Infusions**

Two ml of the appropriate diluted herbal infusions were mixed with 0.1 ml of 1 mmol/L FeCl<sub>2</sub> solution and incubated for 30 before then 0.1 ml of ferrozine was added and further incubated for 10 min [20]. The formed color was measured spectrophotometrically at 562 nm and the results were expressed in EDTA equivalent as average of 3 parallels.

#### **2.7. Color Analyzes and pH measurements of Herbal Infusions**

The color analyzes of herbal infusions were made by the colorimeter (Konica Minolta CM-5, Japan) device and the results are given as lightness (L\*), redness-greenness (a\*), and yellowness-blueness (b\*) in 3 parallels. pH of the infusions was measured with pH (WTW inoLab® pH 7110, Germany).

#### **2.8. Statistical analysis**

Statistical analyses were carried out using Minitab 18 software. The one-way analysis of variance (ANOVA) of data was evaluated, and significant differences were determined between the results with post-hoc test of Tukey's multiple comparison test at p-value  $\leq 0.05$ .

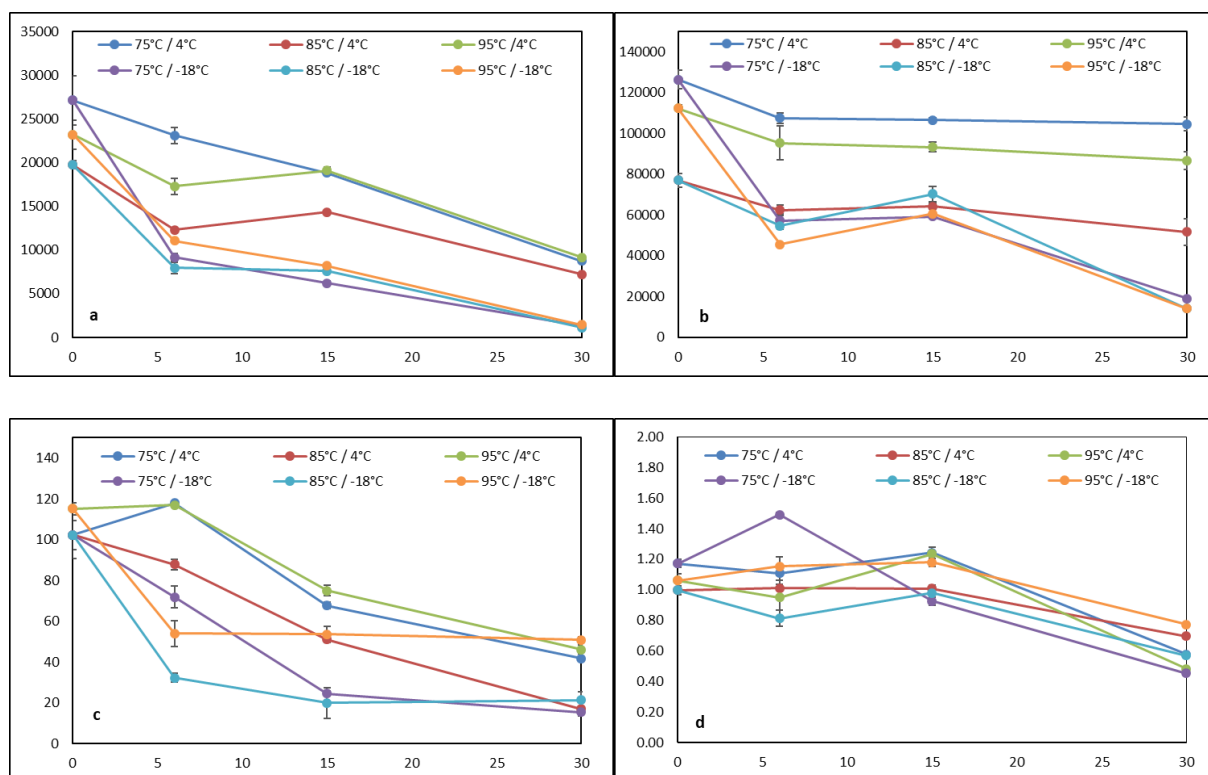
### **3. Results and Discussion**

#### **3.1. Storage stability of cinnamon bark infusions**

The cinnamon bark infusions prepared at 75 (CB75), 85 (CB85), and 95°C (CB95) had  $24349 \pm 2513$ ,  $17720 \pm 419$ , and  $20847 \pm 1486$  µg GA/g dry weight basis TPC, respectively (Figure 1). Although it was expected that the higher process temperature would extract more phenolic compounds from plant cellular material into infusions by softening the structure and disrupting non-covalent interactions such as H-bonding and electrostatic interactions between phenolics and other molecules, some soluble TPC lost were observed by increasing process temperature, which could be associated with heat sensitivity of those phenolic compounds. During 30-day storage at +4°C, significant decrements in TPC were determined in all cinnamon bark infusions. Sixty-eight percent, 63%, and 60% of TPC lost in CB75, CB85, and CB95, respectively. The highest TPC stability (85% remained of initial TPC) was seen in CB75 after 6-day storage. After 15 days, almost 30% of soluble TPC lost in CB75 and CB85 while 18% lost was in CB95. After 6-day storage, between 52 and 66 % TPC lost in all cinnamon infusions was observed at -18°C which was almost equal to TPC lost at +4°C after 30-day storage.

Like TPC, significant reductions were observed in TFC in cinnamon bark infusions during storage at +4°C. The TFC reductions were significant only between 0-6 days and 15-30 days. The highest TFC was determined in CB75

as  $113 \pm 4$  and followed by CB95 as  $101 \pm 2$  and CB85 as  $69 \pm 3$  mg QC/g. Compared to TPC, TFC of CB infusions were highly stable and considerable decrease in values was not observed during 30-day storage. The most stable TFC was in CB75, which remained 86% of initial TFC after 6 days and 82% of initial TFC after 30 days, whereas it was 81% after 6 days and 70% after 30 days in CB85, which was the most unstable infusion. More dramatic decrements were measured in TFC of infusions stored at  $-18^\circ\text{C}$ . After 30 days, 95% of TPC were lost in all cinnamon infusions. This situation clearly indicated the detrimental effect of freezing and thawing processes on phenolic solubility and stability. On the other hand, an extremely high decrease in TFC values was observed by CB infusions stored at  $-18^\circ\text{C}$ . It sharply reduced from  $113 \pm 4$  to  $17 \pm 0$ , from  $69 \pm 3$  to  $13 \pm 1$ , and from  $101 \pm 2$  to  $13 \pm 1$  mg QC/g in CB75, CB85, and CB95 infusions, respectively. Similar instability was also determined in TPC of frozen CB infusions, which again proved that freezing and thawing process adversely affected the soluble phenolic content. Even after 6 days incubation, 55%, 29%, and 59% of TFC disappeared in CB75, CB85, and CB95, respectively. However, TFC stability was higher than TPC stability in frozen stored CB infusions. On the other hand, antioxidant activity of CB infusions was preserved at  $+4^\circ\text{C}$  for 6 days storage. FRSC values of CB infusions were slightly increased from  $83 \pm 10$  to  $106 \pm 0$  (significant) and from  $103 \pm 3$  to  $111 \pm 1$   $\mu\text{mol Trolox/g}$  (non-significant) in CB75 and CB95, while it was slightly decreased from  $92 \pm 6$  to  $84 \pm 2$   $\mu\text{mol Trolox/g}$  (significant) in CB85, but after 30-day storage, 55%, 84%, and 60% of FRSC were disappeared in CB75, CB85, and CB95, respectively ( $P < 0.05$ ). These reductions were more correlated with TPC reductions.



**Figure 1.** (a) total phenolic content ( $\mu\text{g GA/g}$ ), (b) total flavonoid content ( $\mu\text{g quercetin/g}$ ), (c) free radical scavenging capacity ( $\mu\text{mol trolox/g}$ ), and (d) iron chelating capacity ( $\mu\text{mol EDTA/g}$ ) versus storage days graphs of cinnamon infusions produced at  $75^\circ\text{C}$ ,  $85^\circ\text{C}$ , and  $95^\circ\text{C}$  and stored at (+)  $4^\circ\text{C}$  and (-)  $18^\circ\text{C}$  for 30 days. The results were calculated as dry weight basis.

In frozen infusions, a considerable portion of antioxidants lost after 15-day storage where 74%, 80% and 53% of FRSC were not determined in CB75, CB85, and CB95 infusions, respectively. Interestingly, ICC of CB infusions preserved for 15-day storage at  $+4^\circ\text{C}$  and  $-18^\circ\text{C}$ . However, incredibly low or inconsiderable iron chelating ability was determined in CB infusions. During storage, pH of the CB infusions significantly decreased from  $4.93 \pm 0.17$  to  $4.69 \pm 0.08$  in CB75 and from  $4.74 \pm 0.04$  to  $4.60 \pm 0.04$  in CB 95 at  $+4^\circ\text{C}$  and did not significantly change in CB85 as from  $4.84 \pm 0.06$  to  $4.89 \pm 0.13$  ( $P < 0.05$ ).

Significant decreases measured in the pH values of cinnamon teas stored at different temperatures after 30 days ( $P < 0.05$ ). While the pH values did not change or slightly fluctuate in the first 15 days in both storage conditions, it was observed that the teas reached more acidic values when the storage period was extended to 30 days. The pH values were determined between  $4.43 \pm 0.00$  and  $4.89 \pm 0.13$ . This decrement in the solubility or the deterioration of the structure of phenolic components during storage is observed by color measurements. After 6 days, statistically significant increases were detected in  $L^*$  values of all stored samples, which indicates that the tea samples were lighter ( $P < 0.05$ ). This trend continued to increase as the storage period increased, which is insignificant ( $P < 0.05$ ). Increases in these  $L^*$  values were much greater for cinnamon tea samples stored at  $-18^\circ\text{C}$  than the samples stored in other conditions. While the  $a^*$  redness values measured in the cinnamon tea sample brewed at  $75^\circ\text{C}$  were decreased significantly as the storage time increased, the redness was preserved in other stored samples ( $P < 0.05$ ). The  $b^*$  values of the samples stored also showed significant increases in all samples with the storage time, but these increases are more for the samples stored at  $+4^\circ\text{C}$  ( $P < 0.05$ ).

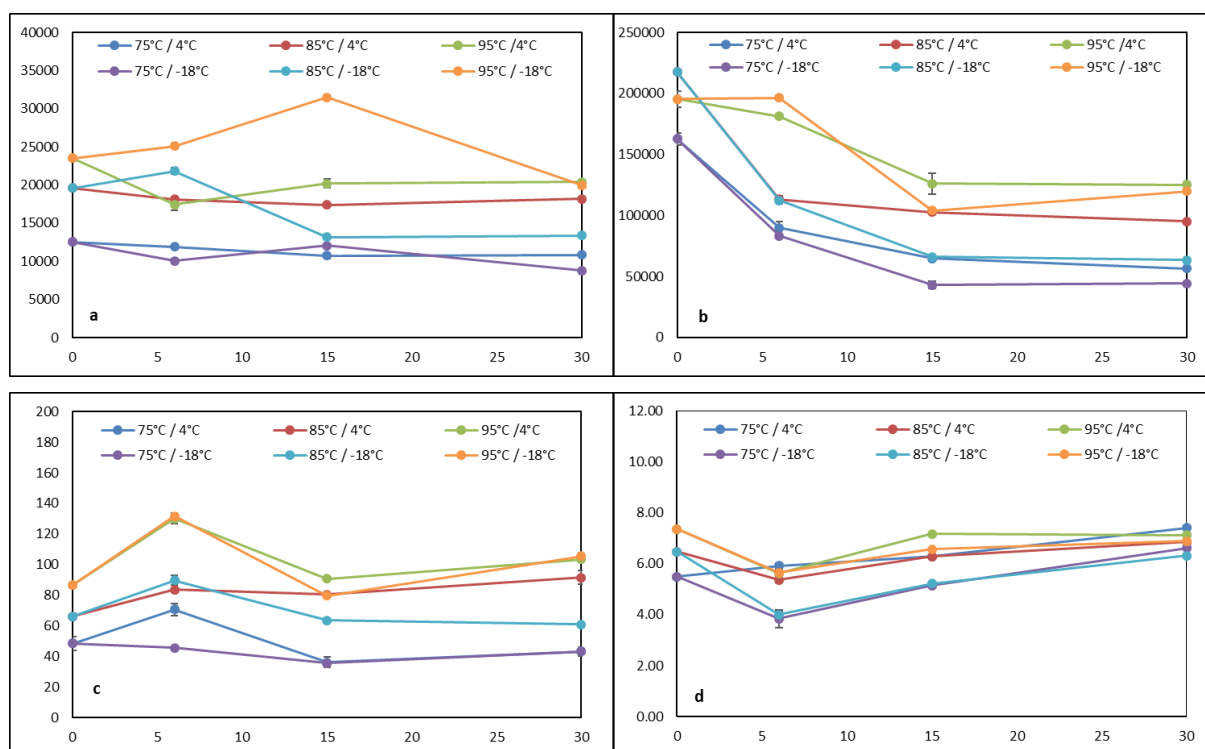
### **3.2. Storage stability of linden flower infusions**

The linden plant consists of flower, leaf and stem parts, and the leaf and flower parts are brewed together or separately for commercial tea production. Linden flower teas produced by brewing at different temperatures stored at  $+4$  and  $-18^\circ\text{C}$  for 30 days, and total water-soluble phenolic and flavonoid contents, antioxidant capacities, pH and color values were measured (Figure 2). The phenolic component contents of the samples did not show a regular increase or decrease depending on the time and the brewing temperature. While significant decreases were observed in TPCs of linden flower tea stored at  $+4^\circ\text{C}$  for the first 15 days, slight increases were observed in 30th day of storage ( $P < 0.05$ ). The highest values for soluble phenolic content at storage are 6th (94% preserved), 30th (93% preserved) and 30th (87% preserved) day for infusions at 75, 85, and  $95^\circ\text{C}$ , respectively. At the end of the 30th day, 86% of the phenolic content of the linden flower tea infused at  $75^\circ\text{C}$  preserved. This indicates that the phenolic components of linden flower teas maintain their stability for a long-time during storage at  $+4^\circ\text{C}$ .

The flavonoid content of the samples stored under the same conditions showed a significant decrease with increasing storage time, unlike the TPC values ( $P < 0.05$ ). Interestingly, this decrease in TFC values is inversely proportional to the brewing temperature. The TFC values of linden flower teas brewed at 75, 85, and  $95^\circ\text{C}$  and stored at  $+4^\circ\text{C}$  decreased by approximately 65%, 56%, and 34%, respectively. This situation indicates that the phenolic components of linden flower teas stored in liquid form at  $+4^\circ\text{C}$  partially decreased in their structure during the first 15 days, and after this time, no decrease was observed in solubility. TPC and TFC values of linden flower teas brewed at different temperatures and stored at  $-18^\circ\text{C}$  by freezing showed significant decreases at the end of 30 days. The loss of phenolic components in tea samples stored by freezing was 2 times higher than tea samples stored as  $+4^\circ\text{C}$ . TPC values increased or decreased irregularly for 30 days. TFC values of the same samples at the same storage temperature decreased with the increasing number of storage days. Among the linden flower teas stored at  $-18^\circ\text{C}$ , the minimum decrease in TPC and TFC values was observed in the tea sample produced by brewing at  $95^\circ\text{C}$ , and a similar situation is generally valid for samples stored at  $+4^\circ\text{C}$ .

Antioxidant properties of linden flower teas followed different trends in both parameters. ICC values of teas either preserved their activities or showed significant increases during the 30-day storage period ( $P < 0.05$ ). The highest increases were 35% ( $6.58 \pm 0.01 \mu\text{mol EDTA} / \text{g}$ ) and 21% ( $6.08 \pm 0.01 \mu\text{mol EDTA} / \text{g}$ ) of teas steeped at  $75^\circ\text{C}$  for 30 days at  $+4^\circ\text{C}$  and  $-18^\circ\text{C}$ , respectively. FRSC values of linden flower teas also decreased slightly or increased significantly after 30 days of storage. FRSC of teas brewed at high temperatures ( $85^\circ\text{C}$  and  $95^\circ\text{C}$ ) increased between 19% and 38% over time, regardless of whether they were stored in at  $+4^\circ\text{C}$  and  $-18^\circ\text{C}$ . Interestingly, the FRSCs of these samples measured after 6 days of storage had the highest values (52%). In the first 6 days, antioxidant substances that can neutralize free radicals can be released into the environment due to depolymerization (Alara et al, 2021).

The pH values of linden flower teas decreased with increasing brewing temperature and storage time, and the samples became more acidic. When the color values were examined, the decrease observed in the  $L^*$  values with the increase of the brewing temperature. While no significant changes were determined in  $a^*$  values,  $b^*$  values increased with the storage time at  $+4^\circ\text{C}$ . The significant decreases were observed in the  $b^*$  values of the samples stored at  $-18^\circ\text{C}$  on the 6th and 15th day ( $P < 0.05$ ).



**Figure 2.** a) total phenolic content ( $\mu\text{g GA/g}$ ), b) total flavonoid content ( $\mu\text{g quercetin/g}$ ), c) free radical scavenging capacity ( $\mu\text{mol trolox/g}$ ), and d) iron chelating capacity ( $\mu\text{mol EDTA/g}$ ) versus storage days graphs of linden flower infusions produced at 75°C, 85°C, and 95°C and stored at (+) 4°C and (-) 18°C for 30 days. The results were calculated as dry weight basis.

### 3.3. Storage stability of linden leaf infusions

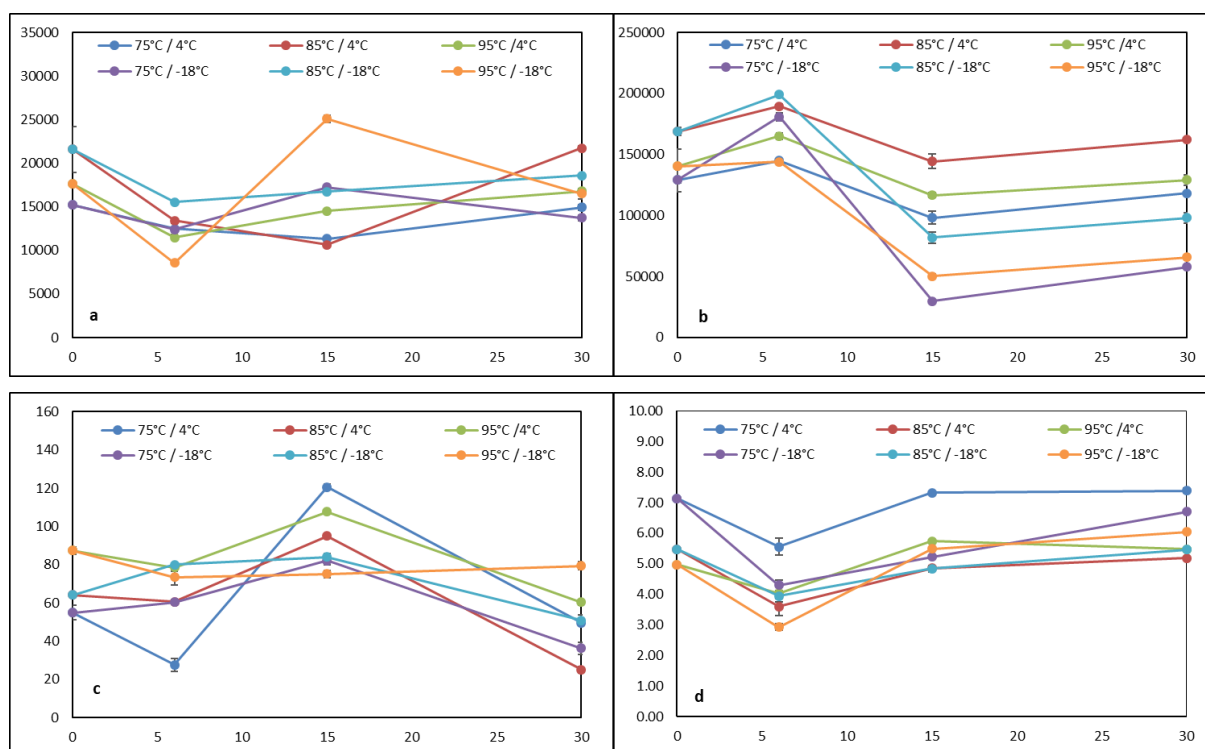
A significant increase in the TPC, FRSC, and ICC of linden leaf tea brewed at elevated temperature. The linden leaf tea stored +4°C and -18°C preserved their TPC between 86% and 100% at the end of 30-day storage (Figure 3). A similar observation was done for TFC of the samples stored for 30 days at + 4°C, which was between 92% and 96% of the initial TFC. TFC of linden leaf teas samples stored at -18°C decreased by approximately 50% after 30 days. Interestingly, the flavonoid contents of these teas reached their highest levels on day six of both storage conditions. While the TFC of all linden leaf teas stored +4°C increased by 12% on the 6<sup>th</sup> storage day. The antioxidant activities of these teas followed a different path from the TPC behaviors, although the phenolic content of teas decreased during storage, or the flavonoid amounts reached their highest values after 6 days and then decreased. While the FRSC of linden leaf teas stored at +4°C decreased in the first days of storage. They reached their highest FRSC value on the 15th day (except linden leaf tea brewed at 95°C and stored at -18°C). These increases were higher in teas brewed at lower temperatures. ICCs of the samples were also preserved during storage. Due to the physical and chemical properties of linden leaf tea, the increment in TPC and ICC were determined during the storage. This high stability of linden leaf tea ingredients, regardless of the brewing temperature and storage condition, indicates that linden leaf tea has the potential to be a natural additive to prevent or delay oxidation of liquid or frozen products.

In both storage conditions of linden leaf teas, pH values decreased with increasing storage time and tea samples became more acidic. While there is a slight decrease in the L\* values of tea stored at + 4 °C during the storage process, there is a slight increase in a\* values over time, while the increase in b\* values is more evident. The yellowish color of linden leaf teas that stored +4°C by time increased, which might be caused by the phenolic components that were oxidized and become dark.

### 3.4. Storage stability of sage leaf infusions

Unlike other tea samples, the limited increments were determined in TPC, TFC, FRSC and ICC values of sage leaf teas brewed at elevated temperatures. During the 30-day storage of sage leaf teas produced by brewing at 75,





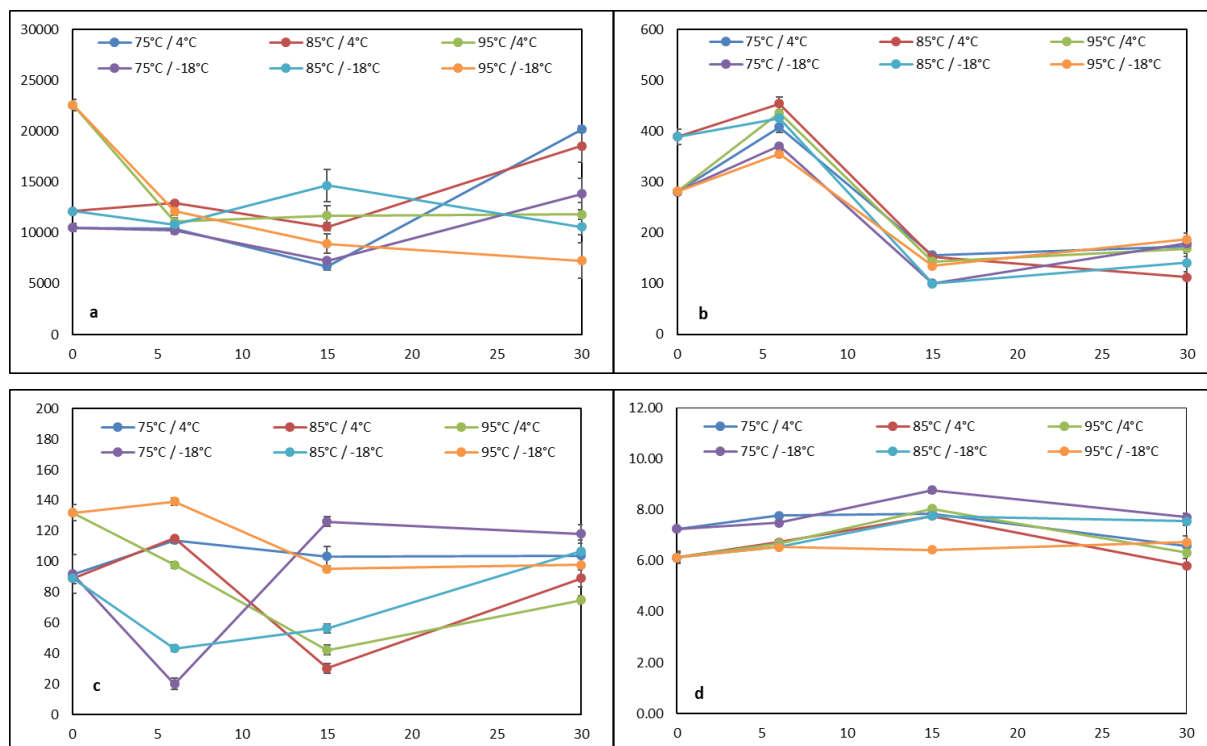
**Figure 3.** a) total phenolic content ( $\mu\text{g GA/g}$ ), b) total flavonoid content ( $\mu\text{g quercetin/g}$ ), c) free radical scavenging capacity ( $\mu\text{mol trolox/g}$ ), and d) iron chelating capacity ( $\mu\text{mol EDTA/g}$ ) versus storage days graphs of linden leaf infusions produced at 75°C, 85°C, and 95°C and stored at (+) 4°C and (-) 18°C for 30 days. The results were calculated as dry weight basis.

85 and 95 °C, significant changes were observed in TPC, FRSC, ICC, pH and color values of teas stored at +4 and -18 °C (Figure 4). Especially, the TPC, FRSC, and ICC of teas brewed at 75 and 85 °C were either preserved or increased up to 1.9 times at the end of 30-day storage in both studied conditions. TPC, FRSC and ICC values of teas were  $12219 \pm 1344$  and  $17830 \pm 283 \mu\text{g GA/g}$ ,  $79 \pm 5$  to  $104 \pm 6 \mu\text{mol trolox/g}$  and  $5.13 \pm 0.02$  to  $6.81 \pm 0.13 \mu\text{mol EDTA/g}$ , respectively. As a result of the storage of teas brewed at 95 °C in both conditions, TPC and FRSC values decreased from 50% to 70%, and from 30% to 40%, while ICC values remained at similar values. Chohan et al. (2008) found that the total antioxidant capacity of the sage (*Salvia fruticosa*) plant was  $625 \pm 0.5 \mu\text{mol trolox/g}$  in their study, in which they investigated the effect of cooking and storage processes on the antioxidant capacity of some edible plants. At the end of 30 days, a significant part of TFC of sage leaf teas stored at +4°C and at -18°C were lost, but the highest TFC values reached in the 6th day measurements of all tea samples. From the 6th to the 15th day, dramatic decreases in TFC values were obtained. In this case, it is thought that the solubility of flavonoids in tea during storage may have increased in the first days due to depolymerization, and then there may be partial decomposition in the structural integrity or solubility due to oxidation or polymerization (Alara et al. 2021).

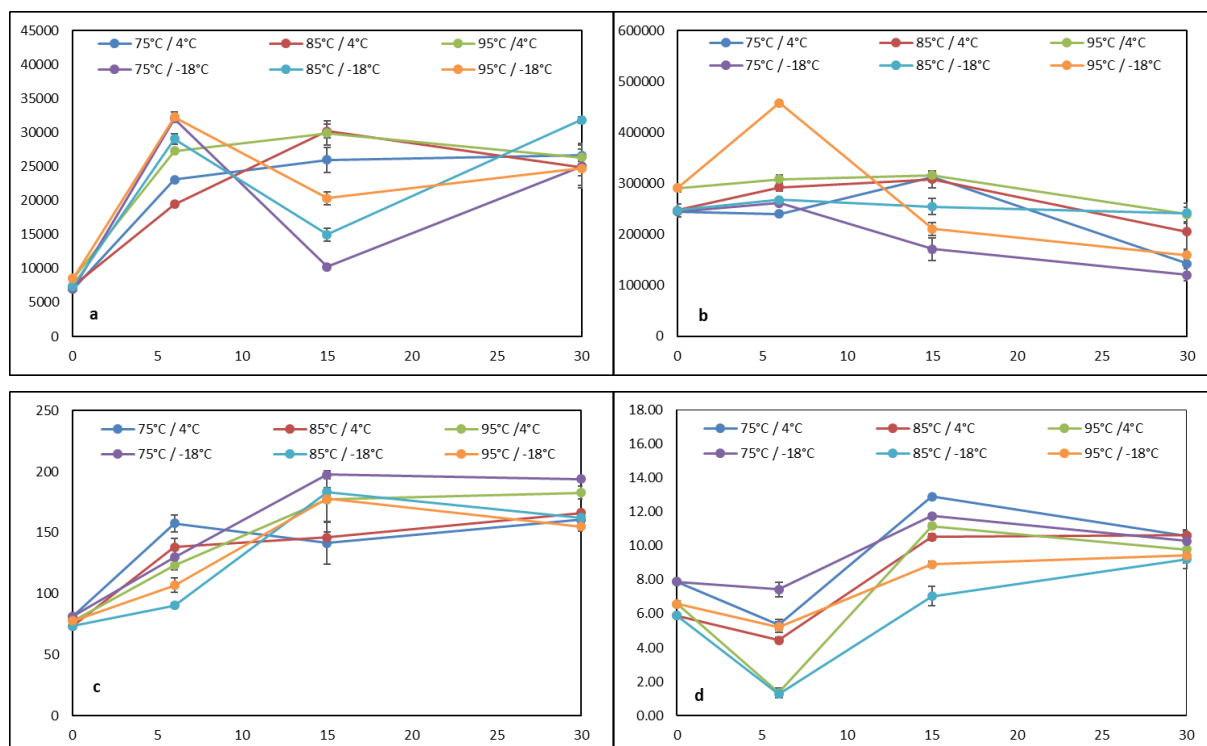
While the pH values of sage leaf teas brewed at different temperatures decreased significantly during storage at +4 °C, they showed more limited changes during storage at -18°C. The L\*, a\* and b\* values of teas brewed at 75°C changed very slightly in both storage conditions, while remarkable decreases in L\* values and significant increases in a\* values of teas brewed at 85°C and 95 °C were determined.

### 3.5. Storage stability of sage stem infusions

TPC and TFC contents of sage stem teas brewed at different temperatures showed significant increases when compared with the samples produced at 25°C (13-15 and 34-41-fold, respectively). No significant increase was observed in FRSC value, while ICC values increased (Figure 5). However, the teas were more acidic with a significant decrease in the pH value. According to the color measurements, the teas were darker, less reddish, and more yellowish ( $P < 0.05$ ). The sage teas brewed at different temperatures and stored at +4°C and -18 °C for 30 days preserved their TPC value while significant increases were observed in the FRSC and ICC. TPC values of sage teas stored at +4 °C varied between  $22217 \pm 2362$  and  $23809 \pm 1546 \mu\text{g GA/g}$  at the end of 30 days, while the



**Figure 4.** a) total phenolic content ( $\mu\text{g GA/g}$ ), b) total flavonoid content ( $\mu\text{g quercetin/g}$ ), c) free radical scavenging capacity ( $\mu\text{mol trolox/g}$ ), and d) iron chelating capacity ( $\mu\text{mol EDTA/g}$ ) versus storage days graphs of sage leaf infusions produced at 75°C, 85°C, and 95°C and stored at (+) 4°C and (-) 18°C for 30 days. The results were calculated as dry weight basis.



**Figure 5.** a) total phenolic content ( $\mu\text{g GA/g}$ ), b) total flavonoid content ( $\mu\text{g quercetin/g}$ ), c) free radical scavenging capacity ( $\mu\text{mol trolox/g}$ ), and d) iron chelating capacity ( $\mu\text{mol EDTA/g}$ ) versus storage days graphs of sage stem infusions produced at 75°C, 85°C, and 95°C and stored at (+) 4°C and (-) 18°C for 30 days. The results were calculated as dry weight basis.



values of teas stored at -18°C varied between  $22086 \pm 916$  and  $28453 \pm 399$   $\mu\text{g GA} / \text{g}$ . during storage. Unlike the TPC values, significant decreases in TFC values were detected during storage, and these reductions were from 17% to 41% and from 2% to 51% at the end of 30 day-storage for tea samples stored at +4 and -18°C, respectively. However, the maximum TFC values were at 15th day of storage at + 4°C. It was seen that the increase in the FRSC of tea samples stored at + 4°C had a positive correlation with storage time and brewing temperature. FRSC values of sage stem teas increased approximately 2.0, 2.3 and 2.4 times for tea samples steeped at 75, 85 and 95 ° C at the end of 30 days, respectively. The increase in antioxidant activity continued and reached the maximum level at the 30th day. The FRSCs of the sage stem samples stored at -18°C reached their maximum values at the 15th day of storage and then slightly decreased.

Sage stem teas had lower pH values during storage, and it was seen that this decrease was more in teas stored at + 4 °C. While the  $L^*$  values of teas stored at -18 °C increased in 30 days of storage, no significant changes were observed in the  $L^*$  values of teas stored at + 4 °C. The  $b^*$  values of teas stored at -18 °C decreased significantly the yellowish colors of teas disappeared. When evaluated together with other results, it is thought that the lightning and yellowish color of the stored teas may be related to the soluble flavonoids found in teas.

#### **4. Conclusions**

Different herbs have been used for centuries in many different societies as a source of pharmaceuticals. Some of them also have been consumed in different ways; brewing, decoction, and infusions are the most applied ways to get benefit from them. The infusion process provides better phenolic and antioxidant extraction from the herbs and their different parts as flower, bark, stem, etc. The infusion temperature has a significant impact on the extraction yield where the higher temperature extracted higher amounts of the compounds. On the other hand, different storage temperatures changed the antioxidant and phenolic content of the infusions in similar trends. These results were interesting since the freezing was expected to preserve the phenolic compounds and antioxidant activity in infusions was better than storing them in a cooled environment. The best antioxidant preservation was observed in linden flower, linden leaf, and sage leaf infusions. However, the phenolic and antioxidant activity decreased in cinnamon infusions. This study opens a perspective to benefit from some medicinal and aromatic herbs to be used as natural antioxidant sources.

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#### **Authors' Contributions**

**LYA:** Conceptualization, Methodology, Resources, Writing - Review & Editing, Supervision, Project administration, Funding acquisition. **FGA:** Validation, Formal analysis, Investigation, Data Curation, Writing - Original Draft, Visualization.

#### **Declaration of Ethical Standards**

The author(s) of this article declare that the materials and methods used in this study do not require ethical committee permission and/or legal-special permission.

#### **Conflict of Interest**

There is no conflict of interest in this study.

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